How to Cite:
Yadav, S. M., Sharma, V. K., Sharma, P. K., & Sharma, J. (2022). Pharmacognostical, phytochemical, antimicrobial and hepatoprotective screening of some plants of family rhamnaceae. *International Journal of Health Sciences*, *6*(S3), 4890–4911. https://doi.org/10.53730/ijhs.v6nS3.6985

Pharmacognostical, phytochemical, antimicrobial and hepatoprotective screening of some plants of family rhamnaceae

Sanwar Mal Yadav
Department of Pharmaceutical Sciences, Apex University Jaipur, Rajasthan, India

Prof. Dr. Vijay Kumar Sharma
Department of Pharmaceutical Sciences, Apex University Jaipur, Rajasthan, India

Prof. Dr. Pankaj Kumar Sharma
Department of Pharmaceutical Sciences, Apex University Jaipur, Rajasthan, India

Prof. Dr. Jaya Sharma
Department of Pharmaceutical Sciences, Apex University Jaipur, Rajasthan, India

***Abstract*---**Medicinal plants are used in traditional system of medicine to treat various diseases and many of these plants have been evaluated for their different pharmacological activities. Ziziphus mauritiana(ZM) (Rhamnaceae) is found wild and cultivated in many parts of India, Burma and Srilanka. Seeds of Ziziphus mauritianahave been reported to have sedative-hypnotic, hypotensive, antihypoxic, antihyperlipidemic and hypothermic activities. It is reported to contain minerals, alkaloids, flavonoids, sterols, tannins and saponins. Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions. The philosophies of these traditional medicines have some resemblance to each other but differ widely from modern western medicine. In view of the progress of western medicine not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine. The development procedure of herbal drugs for world-wide use has to be different from that of synthetic drugs.

***Keywords*---**Ziziphusmauritiana, antioxidant, anti-inflammatory, nephroprotective.

Introduction

The bark of *Ziziphusmauritiana* and *Ziziphusnummularia*used for the present studies were collected from local area of Jaipur district of Rajasthan. The plants were identified, confirmed and authenticated by comparing with voucher specimen available by taxonomist. A copy of certificate is attached. The bark was cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

Material and Methods

Drugs

Indomethacin (Microcid from MicroLabs), Cisplatin (Kemoplat from Dabur)

Plant

The bark of *Ziziphus mauritiana*was procured and authenticated from Regional Research Institute (Ay.) (Central Council of Research in Ayurveda and Sidha, Dept of AYUSH, Ministry of Health and Family Welfare, Govt. of India, New Delhi), Government Central Pharmacy, Annexe, Ashoka Pillar, Jayanagar, Bangalore-560011.

Reagents

Benedict' sreagent, Barfoed' sreagent, Millon's reagent, Dragendroff's reagent. Hagers reagent, Mayer'sreagent. Wagner's reagent.

Chemicals

All chemicals used were of analytical grade.

Collection of plant materials

Bark of the two plants (*Z. mauritiana*Lam. and *Z. nummularia*) were collected in polythene bags from in and around local area of Jaipur-Rajasthan and air dried for two weeks in the Microbiology Laboratory. The dried leaf material was then ground into powder using blender (Monlinex 530, 240V) and packed in polythene bags for further use.

Phytochemical Screening
Preparation of Methanolic Extract

The powdered drugs were dried and packed well in separate Soxhlet apparatus and extracted with 1500 ml of methanol for seven days. The extracts were concentrated and dried using Rotary flash evaporator. Both were kept in desiccators until used.

Qualitative phytochemical screening:[76]

The following tests were carried out on the standardized herbal extracts to detect various phyto-constituents present in them.

Detection of carbohydrates

Small quantity of the extract was dissolved in distilled water and filtered. The filterate was subjected to

- Molisch's test
- Fehling's test
- Barfoed'stest

Molisch's test

To the filterate few drops of alcoholic α-napthol was added and 2ml of conc. sulphuric acid was added slowly through the slides of the test tube. No purple-colored ring was formed at junction of the two layers, which indicates absence of carbohydrates in both the extracts.

Fehling's test

Small portion of the extract was treated with fehling's solution I and II and then heated on water bath. No brick red colored precipitate was formed, which indicates absence of carbohydrates in both theextracts.

Barfoed's test

Small portion of the extract was treated with barfoed's reagent. No red precipitate formed, which indicates absence of carbohydrates in both the extracts.

Test of starch

A small amount of powdered drug was treated with diluted iodine solution. No blue color was observed, which indicates absence of starch in both the drugs.

Detection of proteins and amino acids

Small quantity of extract was dissolved in few ml of water and was subjected to million's, biuret and ninhydrin test.

Million's test

The extract was treated with million's reagent. No white precipitate was produced, shows the absence of proteins and free amino acids in both the extracts.

Biuret test

To the extract equal volume of 5%w/v NaOH and four drops of 1%w/v $CuSO_4$ solution were added. No pink or purple color was formed indicating the absence of proteins in both the extracts.

Ninhydrin test

The extract was treated with ninhydrin reagent. No purple color was produced, indicating the absence of proteins in both the extracts.

Detection of phenolic compounds andtannins

The decoction was diluted with distilled water and filtered. The filtrates were treated with following reagent.

Ferric chloride test

The filtrate was treated with 5% of ferric chloride solution. No black precipitate was found in the decoction of the plant, indicating the absence of tannins and phenolic compounds in both the filtrates.

Test with Lead acetate Solution

Few ml of filtrate was treated with lead acetate solution. No white precipitate was produced in the decoction of both the plants.

Gelatin test

To the filtrate of decoction, add 1ml of 1% solution of gelatin. No white precipitate was seen, which indicates absence of tannin in both the plants.

Test for phytosterols

Small quantity of decoction were dissolved in 5ml of chloroform separately.
Then these chloroform layer subjectedto,

- Salkowskitest
- Libermann – Burchards test

Salkowskitest

To 1ml of the above prepared chloroform solutions, few drops of conc H_2SO_4 was added. Red color produced in the lower layer, shows the presence of phytosterols in both the samples.

Libermann – Burchardstest

The above chloroform solution was treated with few drops of conc. H_2SO_4 followed by 1ml of acetic anhydride solution. Green color was produced, shows the presence of phytosterols in both the solutions.

Test for fixed oils and fats

3Spot test

A small quantity of extracts was pressed between two filter papers, oil stain was observed in both the extracts, show presence of fixed oils in both the samples.

Saponification

Few drops of 0.5N alcoholic potassium hydroxide was added to extracts along with a few drops of phenolphthalein. The mixture were heated on a water bath for about 1 – 2 hours. Formation of soap or a partial neutralization of alkali indicated the presence of fixed oils andfats in both the samples.

Test for alkaloids

Small amount of extracts were stirred with a few ml of dil.HCl and filtered. The filtrates were tested with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

Mayer's test

To the small amount of filtrates few drops of Mayer's reagent was added. A white color precipitate was formed, indicating the presence of alkaloids in both.

Dragendroff's test: (potassium bismuthiodide)

To the small amount of filtrates few drops of Dragendroff's reagent was added. An orange red color precipitate was formed in both the samples, indicating the presence of alkaloids in both the samples.

Wagner's test

To the small amount of filtrate of both the drugs few drops of Wagner's reagent was added. A brown color precipitate was formed in both, indicating the presence of alkaloids in both the samples.

Hager's test: (picric acid)

To the small amount of filtrate of both drugs few drops of Hager's reagent was added. A yellow crystalline precipitate was formed in both samples, indicating the presence of alkaloids in both the samples.

Test for glycosides

Small amount of the extracts were hydrolyzed separately with hydrochloric acid for one hour on separate water baths and hydrolysates were subjected to.

Legal's test

To the hydrolysate 1ml pyridine few drops of sodium nitroprusside solution was added and then made alkaline with NaOH solution. Pink color was obtained showing the presence of glycosides in both the samples.

Balget's test

To a solution of extract sodium picrate solution was added. Yellowish orange color was obtained showing, the presence of glycosides in both the samples.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Pink color was observed in ammoniacal layer, confirms the presence of glycosides in both the samples.

Modified borntrager's test

The extracts were boiled with few ml of dil. HCl and 5ml of ferric chloride solution. The contents are cooled and shaken with organic solvent. Organic layer was separated and to this equal volume of ammoniacal solution was added. The ammoniacal layer showed pink color. In this test, addition of ferric chloride was addedto break the C – C linking of glycosides which is a stronger than C = O linkage in both the samples.

Test forflavonoids

The extracts were dissolved in ethanol separately and then subjected to the following tests.

Ferric chloride test

To a small quantity of Methanolic solution of extract few drops of neutral ferric chloride was added. Blackish red color was observed, showing the presence of flavonoids in both the samples.

Shinoida's test

To the alcoholic solution a small piece of magnesium ribbon was added along with conc. HCl. Magenta color was formed, showing the presence of flavonoids in both the samples.

Fluorescence test

Alcoholic solution was seen under ultra violet light. Green color fluorescence was observed, indicating the presence of flavanoids. Reaction with alkali andacid: With sodium hydroxide solution the extracts gave yellow color. Extract gave orange color with conc H_2SO_4 indicating the presence of flavonoids in both the samples.

Zinc, HCl reduction test

To a small quantity of extract, a pinch of zinc dust was added. Then add few drops of conc. HCl. Magenta color was produced, the presence of flavonoids in both the samples.

Lead acetatesolution

To a small quantity of extract a few drops of 10% lead acetate solution was added. Yellow precipitate was produced, shows presence of flavonoids in both the samples.

Detection of saponins

The extracts were diluted, with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. A one centimeter layer of foam was formed, indicating the presence of saponins in both the samples.

Detection of coumarins

To a small quantity of extract were dissolved in alcohol and exposed to UV light, shows green fluorescence. To small quantity of extract were dissolved in alcohol and add ferric chloride solution, shows green color, indicating the presence of coumarins in both the samples.

Extraction of active compounds using ethanol as solvent for extraction

Ten grams (10g) of the ground barks samples were separately soaked in 200 ml of ethanol and allowed to stand for about 72 h for extraction. After the 72 h, it was then filtered using No.1 Whatman filter paper. The filtered samples were sterilized by passing through Millipore filter and later evaporated to dryness (Mann et al., 2008).

Preparation of test organisms

Clinical specimens of *Staphylococcus aureus, E. coli, S. pyogenes,C.albicans*and*A. niger*were obtained from Advance Labs,Mansarovar, Jaipur. The organisms were sub - cultured on agar slants prior to use. 18 h liquid culture of each of the organisms was used for sensitivity testing.

Sensitivity testing

Ethanolic extract (preparation is shown above) of each plant sample was tested against each of the organisms using agar cup well method as described by Okeke et al. (2001). After making holes with No. 4 cork borer, the surface of the agar was lawned with18 h culture of the test organism which has been previously standardized to 10^6. Same volume (0.1ml) of different concentrations of the extract (500, 50, 5 and 1 mg) was dropped with the aid of dropper pipette into each well. The plates were incubated at 37°C for 24 h and 72 h at 25°C for bacteria and fungi respectively.

Antioxidant activity
DPPH (1,1-Diphenyl-2-picryl-hydrazil) free radical scavengingactivity:[62]

DPPH radical scavenging activity of *Ziziphus mauritiana*(aqueous and ethanolic extracts) was determined by the method described by *Shimada et al.* One ml of 0.1mM ethanolic solution of DPPH was added to 3 ml of *Ziziphus mauritiana*aqueous and ethanolic extracts of different concentrations (50, 75, 100,150,200µg/ml each of ethanolic and aqueous extracts). The mixture was shaken vigorously and allowed to stand at room temperature for 30min. Then the absorbance was measured at 517nm by using spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging effect.

Nitric oxide radical scavengingactivity:[23]

Nitric oxide generated from sodium nitroprusside (SNP) was measured using griess reagent. The reaction mixture (3ml) containing100mM SNP (0.2ml, final concentration 10mM) and PBS (1.8ml) and extracts at various concentrations (1 ml) was incubated at 25^0c for 180 min. At the end of 30 min, 1ml of incubated solution was mixed with 1ml of griess reagent (1%sulphanilamide and 0.1% napthyletylenediamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthyletylenediamine was read at 540nm.

Superoxide radical scavengingactivity:[14]

Measurement of superoxide anion radical scavenging activity of *Ziziphus mauritiana*was based on the method described by Liu et al. Superoxide radicals generated in PMS-NADH systems by oxidation of NADH was assayed by the reduction of NBT. To 1 mlof test solution (25, 50, 100, 150, 200µg/ml), 1ml of phenazine methosulphate (PMS, 60µm in phosphate buffer) and 1ml of NADH (450 µm in phosphate buffer) was added. At the end, 1ml of nitroblue tetrazolium (NBT, 300 µm in ethanol) was added. The reaction mixture was incubated at 25^0c for 5min and absorbance was measured at 560nm using spectrophotometer. Absorbance of control (all the reagents without test solution under similar conditions) was also recorded. All the experiments were conducted in triplicate. Radical scavenging activity was calculated using the following equation.

% Radical scavenged = $(A_0 – A_{1)}/ A_0 \times 100$. Where, A_0 = Absorbance of control
A_1 = Absorbance of test solution or standard.

Pharmacological studies
Hepatoprotective activity

For the evaluation of hepatoprotective activity of *Ziziphusmauritiana*and *Ziziphusnummularia* against paracetamol-induced liver injury in experimental rats, The experiments were performed on albino male Wistar rats, 2 months old, weighing 170 ± 15 g. The animals were maintained under the following laboratory conditions: lights on 06.00–18.00; 60% relative humidity; 22° ± 2° C room temperature, access to commercial food pellets, and tap water ad libitum. 42 rats were used and randomly assigned to 7 groups (6 rats per group) and treated orally with the following compounds for 30 consecutive days: Group A -Normal control (1%) (10 ml/kg body weight) Group B - served as hepatotoxic control Toxicant (3.76 gm/kg of Ethanol, twice daily, p.o) Group C - served as standard drug treatment group and received silymarin (25 mg/kg) Group D - Methanolic extract of *Ziziphusmauritiana*bark 200mg/kgtwice daily, p. o Group D1 - Methanolic extract of *Ziziphusnummularia*bark 200mg/kgPCM twice daily, p. o Group - E Methanolic extract of *Ziziphusmauritiana*bark 400mg/kgtwice daily, p. o *Group* E1 - Methanolic extract of *Ziziphusnummularia*bark 400mg/kg PCM twice daily, p. o PCM was administered 1 h before the administration of either silymarin, *Ziziphusmauritiana*bark or *Ziziphusnummularia*bark. In the end, all animals were euthanized with ether overdose, and blood collected by retro orbital puncture into plain dry tubes and centrifuged at 2000 rpm for 10 min. The serum samples obtained were transferred into eppendorf tubes and analyzed biochemical parameters like ALT, AST, ALP, Direct Bilirubin, Total Bilirubin, Triglycerides, Cholesterol, Total Proteins and Albumin were estimated[76]. The animals were sacrificed and autopsied. Livers from all animals were removed, washed with ice-cold saline, weighed and measured the wet liver volume. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. Livers of some animals were homogenized with ice-chilled 10% KClsoln and centrifuged at 2000 rpm for 10 minutes. Then the supernatant liquid was collected and the antioxidant parameters like Catalase, Super oxide Dismutase and Thiobarbiturate were estimated[76].

Histopathological Studies
Processing of isolated liver

The animals were sacrificed and the liver of each animal was isolated and was cut into small pieces, preserved and fixed in 10% formalin for two days. Then the liver piece was washed in running water for about 12 hours to remove the formalin and was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with about three changes for 12 hours each. Dehydration was performed to remove all traces of water. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration. The clearing was done by using chloroform with two changes for 15 to 20 minutes each. After paraffin infiltration the liver pieces were subjected to automatic tissue processing unit. Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allow to cool. The blocks were cut using microtome to get sections of thickness 5 µ The Sections were taken on a microslide on which egg albumin i.e., sticking substance

was applied. The sections were allowed to remain in an oven at 60^0C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissue to slide.

Result

Phytochemical constituents present in Methanolic extract of bark ofZiziphusmourtiana

Table 1
Details of qualitative phytochemical tests

S.No.	Test	Pet-etherExtract	ChloroformExtract	MethanolicExtract
1	Carbohydrates			
	Mohlish's test	-	-	+
	Fehling's test	-	-	-
2	Proteinsandaminoacids			
	Ninhydrin test	-	-	-
	Biuret test	-	-	-
3	Alkaloids			
	Mayer's test	-	+	+
	Wagner's test	-	-	+
4	Fixed oils and fats			
	Spot test	+	-	-
5	Glycosides			
	Borntrager's test	-	-	+
	Legals test	-	+	+
6	Triterpenoids			
	Tin + thionyl chloride	+	-	-
7	Phenolics and tannins			
	Ferric chloride test	-	-	-
	Gelatin test	-	-	-
	Lead acetate test	-	-	-
	Alkaline reagent test	-	-	-
	Dilute HNO3 test	-	-	-
8	Saponins			
	Foam test	-	+	+
	Haemolysis test	-	+	+
9	FlavonesandFlavonoids Flavonoids			
	Caddy's test	-	+	+
	Shinoda test	-	+	+

s(+) Indicates positive result (–) Indicates negative result.

In, preliminary phytochemical studies of extracts of *Ziziphus mauritiana*confirmed the strong presence of desired phytochemicals in methanolic extracts when compared to pet-ether and chloroform extracts. Hence, for the further studies Methanolic extract of *Ziziphus mauritiana*(MEAL) have beenselected.

Phytochemical constituents present in Methanolic extract of bark of *Ziziphus nummularia*

Table 2
Details of qualitative phytochemical tests

S.No.	Test	Pet ether Extract	Chloroform Extract	Methanolic Extract
1	Carbohydrates			
	Mohlish's test	-	-	+
	Fehling's test	-	-	-
2	Proteins and amino acids			
	Ninhydrin test	-	-	-
	Biuret test	-	-	-
3	Alkaloids			
	Mayer's test	-	+	+
	Wagner's test	-	-	+
4	Fixed oils and fats			
	Spot test	+	-	-
5	Glycosides			
	Borntrager's test	-	-	+
	Legals test	-	+	+
6	Triterpenoids			
	Tin + thionyl chloride	+	-	-
7	Phenolics and tannins			
	Ferric chloride test	-	-	-
	Gelatin test	-	-	-
	Lead acetate test	-	-	-
	Alkaline reagent test	-	-	-
	Dilute HNO3 test	-	-	-
8	Saponins			
	Foam test	-	+	+
	Haemolysis test	-	+	+
9	Flavones			
	Caddy's test	-	+	+
	Shinoda test	-	+	+

(+) Indicates positive result (–) Indicates negative result.

In, preliminary phytochemical studies of extracts of *Ziziphus nummularia confirmed* the strong presence of desired phytochemicals in methanolic extracts when compared to pet-ether and chloroform extracts. Hence, for the further studies methanolic extract of *Ziziphus nummularia*(MEAL) have been selected.

Antioxidant activity

The antioxidant potential of a substance can be evaluated based on its ability to scavenge free radicals like DPPH, nitric oxide and super oxide. Based on this the *in-vitro* antioxidant activity of aqueous and ethanolic extracts of *Ziziphus mauritiana*seeds was evaluated and the results are as follow.

DPPH scavenging activity

Both ethanolic and aqueous extracts of ZM produced scavenging of DPPH radicals from 50µg/ml. The same was observed with the standard antioxidant ascorbic acid. Maximum scavenging of DPPH was observed at 200µg/ml. The percentage of DPPH scavenged at 200 µg/ml by ascorbic acid, ZMA and ZME were found to be 79.02± 0.52, 45.69 ± 0.52, 63.27 ±0.59 respectively. Significant (*P<0.05*) DPPH scavenging activity was shown by ZME at 200µg/ml which was comparable to that of standard ascorbic acid.

DPPH scavenging activity of *Ziziphus mauritiana extracts*

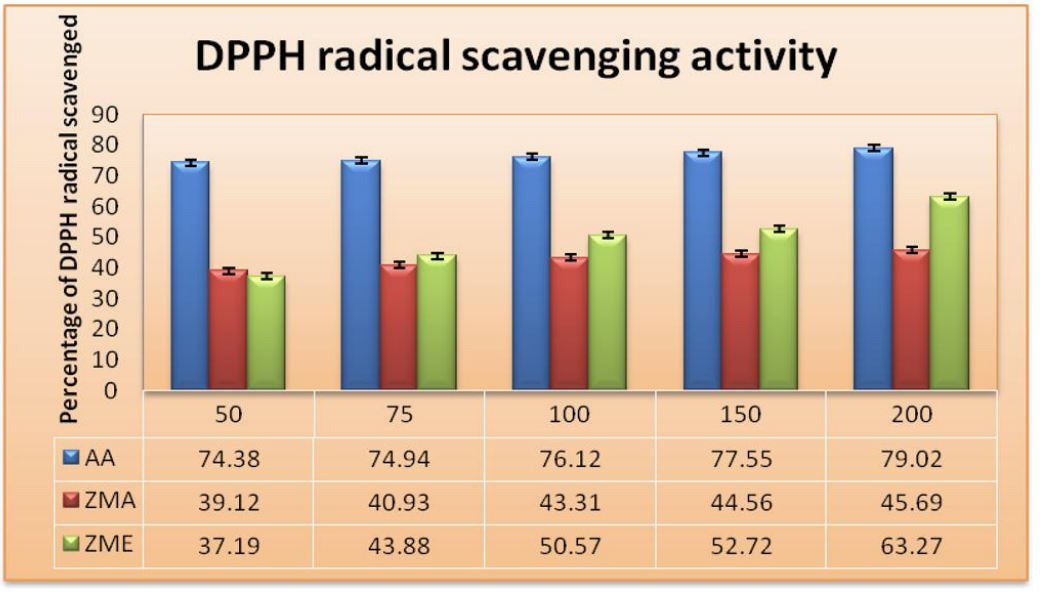


Fig 1. n=3, values are percentage of DPPH radical scavenged, mean ± SD, where ZMA, ZME and AA indicate *Ziziphus mauritiana*aqueous and ethanolic extracts, ascorbic acid respectively

Table 3
Percentage of DPPH radical scavenged at various concentration

Test Compound	Percentage of DPPH radical scavenged at various concentration				
	50	75	100	150	200
AA	74.38±0.19	74.94±0.20	6.12±0.57	77.55±0.34	79.02±0.51
ZMA	39.12±0.34	40.93±0.78	43.31±0.39	44.56±0.0	45.69±0.51
ZME	37.19±0.52	43.88±0.68	50.57±1.96	52.72±0.34	63.27±0.58*a

n=3, values are percentage of DPPH radical scavenged, mean ±SD, where ZMA and ZME indicates *Ziziphus mauritiana*aqueous and ethanolic extracts respectively, AA- Ascorbic acid -**P<0.05*, 'a' indicates comparison of extracts with ascorbic acid.

Nitric oxide scavenging activity

Both ethanolic and aqueous extracts of *Ziziphus mauritiana*produced nitric oxide scavenging activity at the various concentrations 50, 100, 150, 200 and 250µg/ml. When nitric oxide scavenging activity of ethanolic and aqueous extracts of *Ziziphus mauritiana*was compared with standard BHA the following observations were made and tabulated. (Table 6, Figure 3

Nitric oxide scavenging activity of *Ziziphus mauritiana*extracts

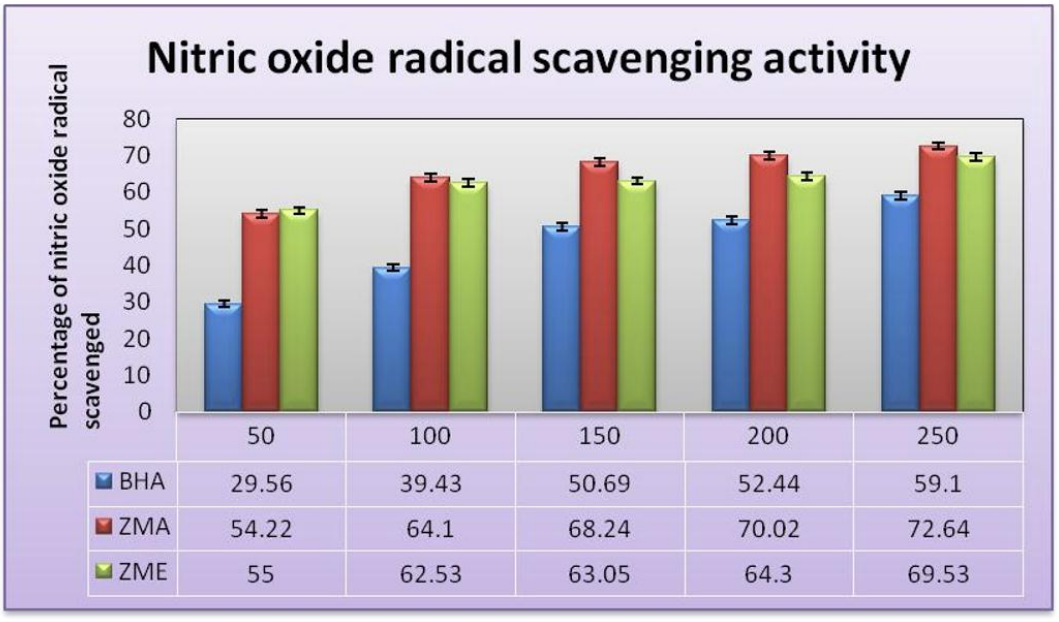


Fig 2. n=3, values are percentage of nitric oxide scavenged, mean ± SD, where ZMA, ZMA and BHA indicates *Ziziphus mauritiana*aqueous and ethanolic extracts, butylated hydroxyl anisole respectively

Both aqueous and ethanolic extracts of *Ziziphus mauritiana*at 50, 100, 150, 200 and 250µg/ml produced significant *(P<0.001)* nitric oxide radical scavenging activity which was better than the standard BHA at all concentrations. ZMA produced maximum nitric oxide scavenging activity at 250µg/ml followed by ZME and BHA (72.64±0.66, 69.73±1.73 and 59.1±1.74 respectively).

Table 4
Percentage of nitric oxide radical scavenged by aqueous and ethanolic extracts of *Ziziphus mauritiana*

Test compound	Percentage of nitric oxide scavenged at various concentration				
	50	100	150	200	250
BHA	29.56±8.53	39.43±3.80	50.69±4.02	52.44±1.07	59.1±1.7
ZMA	54.22±0.49**a	64.1±3.04*** a	68.24±0.90***	70.02±0.91***	72.64±0.65*** a
ZME	55±3.68*** a	62.53±2.95*** a	63.05±1.69** a	64.3±2.69*** a	69.53±1.72*** a

n=3, values are percentage of nitric oxide radical scavenged, mean ±SD, where, ZMA, ZME and BHA indicates *Ziziphus mauritiana*aqueous and ethanolic extracts, butyl hydroxyanisole respectively -
***P<0.01, ***P<0.001*, 'a' indicates comparison of ZMA and ZME with standard-BHA.

Superoxide scavenging activity

Both aqueous and ethanolic extracts of *Ziziphus mauritiana*produced superoxide radical scavenging activity from 25µg/ml and it increased up to 125µg/ml (55.22±1.89 to 63.83±0.77and 67.83±2.90 to 81.82±0.13 respectively). When super oxide radical scavenging activity of ethanolic and aqueous extracts of *Ziziphus mauritiana*was compared with standard BHA the following observations were made and are as follows. The ethanolic extract of *Ziziphus mauritiana*at 25, 50, 75, 100, 125µg/ml produced significant *(P<0.001)* super oxide radical scavenging activity which was better than the standard BHA at all concentrations. Significant (*P< 0.001)* percentage inhibition of superoxide was produced by aqueous extract at 25 and 50µg/ml which was better than that of BHA. ZME produced maximum superoxide activity at 125µg/ml followed by BHA and ZMA (81.82±0.13, 67.47±1.52 and 63.83±0.77 respectively) (Table 7, Figure 4).

Superoxide scavenging activity of *Ziziphus mauritiana*extracts

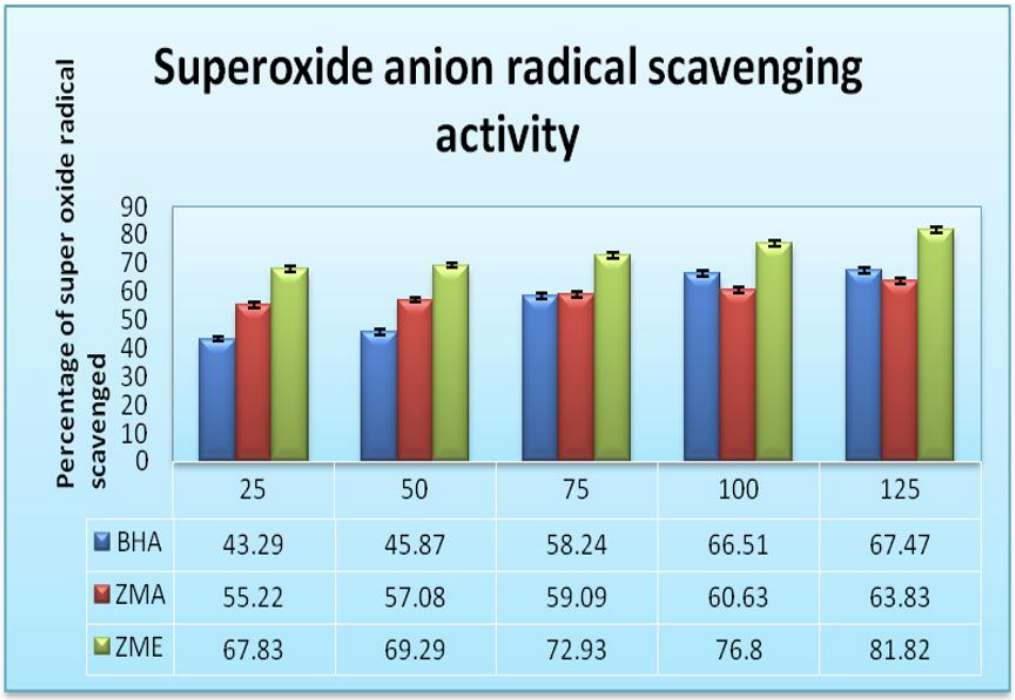


Fig 3. n=3, values are percentage of super oxide scavenged, mean ± SD, where ZMA, ZMA and BHA indicates *Ziziphus mauritiana*aqueous and ethanolic extracts, butylated hydroxyl anisole respectively

Table 5

Percentage of superoxide radical scavenged by aqueous and ethanolic extracts of *Ziziphus mauritiana*

Test compound	Percentage of super oxide radical scavenged at various concentration				
	25	50	75	100	125
BHA	43.29±1.18	45.87±2.41	58.24±0.83	66.51±1.04	67.47±1.52
ZMA	55.22±1.89***	57.08±0.92***	59.09±2.70	60.63±1.77**[a]	63.83±0.77*[a]
ZME	67.83±2.90***[a]	69.29±1.73***[a]	72.93±1.80***[a]	76.8±1.44***[a]	81.82±0.13***[a]

n=3, values are percentage of super oxide radical scavenged, mean ±SD, where ZMA, ZME and BHA indicates *Ziziphus mauritiana*aqueous and ethanolic extracts, Butyl hydroxyl anisole respectively –
****P<0.001,* ***P<0.01*, 'a' indicates comparison of ZMA and ZME with std-BHA.

Wet liver weight and Wet liver volume

In the study treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and methanolic extract of *Ziziphusmauritiana*bark and Ziziphusnummularia showed significant restoration of wet liver weight and wet liver volume nearer to normal. The MEAL at 200mg/kg b.wt and 400mg/kg body weight showed reduction of wet liver weight and wet liver volume significantly at p<0.05.The results are shown in table no.5.2 and fig.5.1 and 5.2.

Table 6
Effect of methanolic extract of Ziziphusmauritiana and Ziziphus nummularia on Wet liver weight and Wet liver volume in Ethanol induced hepatotoxic rats

Group	Treatment	Dose	Wet Liver weight (gm/100gm)	Liver volumes (ml/100gm)
A	Normal control	10ml/kg p.o	2.53 ± 0.535	2.535±0.53
B	Toxicant Control	Ethanol 3.76 mg/kg, p.o.	4.34 ± 0.095	4.19±0.04
C	Standard	200mg/kg, p.o + Ethanol	2.61 ± 0.110*	2.78±0.23*
D1	Ziziphusmauritiana	200mg/kg, p.o + Ethanol	2.73 ± 0.120*	2.973±0.07*
D2	Ziziphusmauritiana	400mg/kg, p.o + Ethanol	2.75 ± 0.124*	2.977±0.05*
E1	Ziziphus nummularia	200mg/kg, p.o + Ethanol	2.36 ± 0.27*	2.77±0.11*
E2	Ziziphus nummularia	400mg/kg, p.o + Ethanol	2.34± 0.24*	2.74±0.14*

Values are mean ± SEM (n=6) one way ANOVA.Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, and *** represents very significant at p<0.001. All p values are compared with toxicant.

Bio chemical Parameters

Effect of methanolic extract of Ziziphus mauritiana and Ziziphus nummularia bark on biochemical parameters in ethanol induced hepatotoxicrats

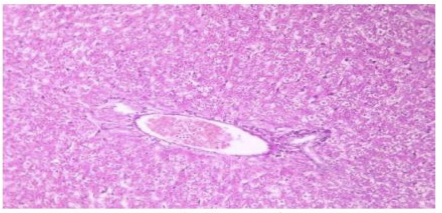
Rats treated with ethanol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and SALP when compared to normal control. Pretreatment with Silymarin, methanolic extract had showed good protection against ethanol induced toxicity to liver. Test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals which is evident in table no.5.3.

Table 7
Effect of methanolic extract of Ziziphus mauritiana and Ziziphus nummularia bark on SGPT, SGOT & SALP levels in ethanol induced hepatotoxic rats

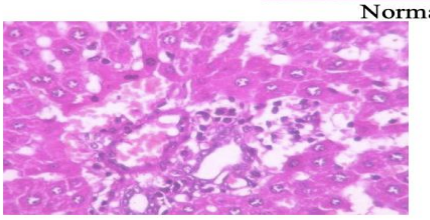
Group	Treatment	Dose	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)	Direct bilirubin levels(mg/dl)	Total bilirubin levels (mg/dl)	Total protein levels (gm/dl)	Cholesterol levels (mg/dl)	Triglyceride levels (mg/dl)
A	Normal control	10ml/kg p.o	29.35±0.90	34.90±1.50	28.15±1.141	0.184±0.0091	0.22±0.02	6.94±0.06	6.42±0.164	29.29±0.451
B	Toxicant Control	Ethanol 3.76 mg/kg, p.o.	123.9±1.50	177.95 ±1.350	81.24±1.388	0.85±0.0303	1.36±0.06	3.47±0.02	32.22±0.547	160.52±2.58
C	Standard	200mg/kg, p.o + Ethanol	50.57±0.05***	86.86±0.7025***	30.8±2.05***	0.35±0.014***	0.43±0.04***	5.74±0.15**	9.84±0.448***	56.43±0.63***
D1	Ziziphusmauritiana	200mg/kg, p.o + Ethanol	85.6±0.55*	112.56±0.750*	64.0±2.05*	0.65±0.02*	1.08±0.07	4.08±0.15**	19.30±0.88*	102.43±0.72*
D2	Ziziphusmauritiana	400mg/kg, p.o + Ethanol	85.4±0.54*	112.54±0.744*	63.4±2.03*	0.43±0.02**	0.71±0.05**	5.11±0.08**	14.23±0.55**	72.56±0.98***
E1	Ziziphus nummularia	400mg/kg, p.o + Ethanol	72.4 ±0.05**	102.3±0.50*	38.6±0.97***	0.66±0.06*	1.06±0.07s	4.05±0.12**	19.31±0.82*	101.44±0.85*
E2	Ziziphus nummularia	400mg/kg, p.o + Ethanol	72.3 ±0.04**	103.4±0.52*	38.4±0.95***	0.47±0.03**	0.74±0.08**	5.18±0.09**	14.27±0.55**	72.52±0.92***

Values are mean ± SEM (n=6) one way ANOVA . Where, * represents significant at $p<0.05$, ** represents highly significant at $p< 0.01$, and *** represents very significant at $p<0.001$. All values are compared with toxicant. Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal (group 1) exhibited normal hepatic cells each with well-defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Figure 1), whereas that of Ethanol induced intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization and congestion of sinusoids, kupffer cell hyperplasia, crowding of central vein and apoptosis (Figure 2). Treatment with methanol extract of two plants, *Ziziphus mauritiana and*

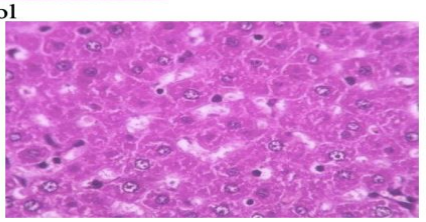
Ziziphus nummulariabark at a dose of 200 and 500 mg/kg b.wt. showed moderate to weak activity in protecting the liver cells from Ethanol -injury (Figure 3 to 6). Among these plant extract, treatment with both plant extract returned the injured liver to quite normal. Now, it could be decided that the hepatoprotective activity was dose and time dependent. Out of four plant extracts, the crude methanol extract had shown very potential heptoprotective activity at a dose of 500 mg/kg b.wt.



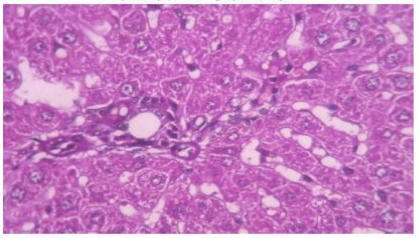
Normal Control



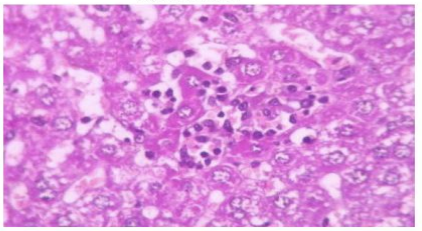
Toxicant Control



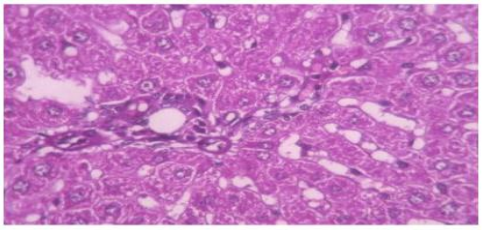
Standard



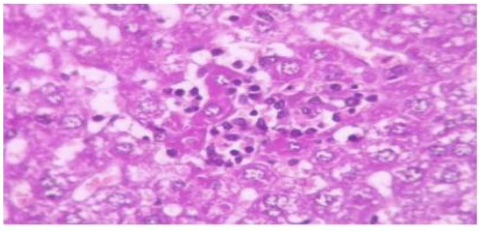
Ziziphusmauritiana 200mg/kg



Ziziphusmauritiana 400mg/kg



Ziziphus nummularia 200mg/kg



Ziziphus nummularia 400mg/kg

Anti-Bacterial Activity

Table 8
Sensitivity analysis showing zones of inhibition (mm) around crude extracts at varying concentrations

Conc. of extracts(mg/ml)	*S.a*	*E.c*	*S.p*	*A.n*	*C.a*	*S.a*	*E.c*	*S.p*	*A.n*	*C.a*
1	0	0	9 ± 3	0	0	0	0	0	0	0
5	0	9 ± 1	14 ± 3	0	0	0	10 ± 4	13 ± 1	0	0
50	11 ± 2	12 ± 2	16 ± 3	0	0	12 ± 3	14 ± 0	18 ± 0	0	0
500	13 ± 1	16 ± 3	26 ± 2	0	0	15 ± 2	17 ± 1	28 ± 2	0	0

Key: *S.a = Staphylococcus aureus, E.c = Escherichia coli, S.p = Streptococcus pyogenes, A.n= Aspergillusniger,* C.a = *Candida albicans.*

Table 9
Zones of clearing (mm) of susceptibility testing with standard antibiotics

Organisms	Antibiotics									
	CH	CPX	E	LC	GM	APX	RP	FLX	S	NB
E. coli	9 ± 0.1	8 ± 0.1	9 ± 0.1	0	12 ±	0	0	9 ±	8 ±	12 ±
S. pyogenes	12 ±	10 ±	9 ± 0.1	8 ± 0.1	11 ±	8 ± 0.1	8 ±	9 ±	9 ±	14 ±
S. aureus	18 ±	18 ±	16 ±	15 ±	16 ±	12 ±	14 ±	12 ±	16 ±	16 ±
A. niger	19 ±	0	0	22 ±	22 ±	18 ±	22 ±	0	16 ±	0
C. albicans	0	0	0	0	0	0	0	0	0	0

Key: CH - Chloramphenicol (10 mg), CPX - Ciprofloxacin (10 mg), E - Erythromycin (20 mg), LC - Lincocin (30 mg), GM - Gentamycin (10 mg), APX - Ampiclox (10 mg), RP - Rimbaprim (10 mg), FLX - Floxapin (30 mg), S - Streptomycin (30 mg), NB - Narbactin (10 mg).

Table 10
Minimum inhibitory concentration (MIC) of plant extracts against test organisms.

Plantparts	Organisms	Concentration of extract(mg/ml)							
		50	40	30	20	5	1	0	MIC
Z. mauritiana	*E. coli*	-	-	-	-	-	+	+	5
	S. aureus	-	-	+	+	+	+	+	40
	S. pyogenes	-	-	-	-	-	-	+	1
Z. nummularia	*E. coli*	-	-	-	-	+	+	+	20
	S. aureus	-	+	+	+	+	+	+	50
	S. pyogenes	-	-	-	-	-	+	+	5

Key: + = Growth, - = No growth.

Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration was determined using tube dilution technique. Varying concentrations of each extract were prepared and 1 ml introduced into 9 ml of nutrient broth in test tubes. About 0.1 ml of the 18 h culture diluted to 10^6 cell ml^{-1} was added and incubated accordingly. The least concentration of the extract that did not permit turbidity in the broth was taken as the minimum inhibitory concentration.

Minimum bactericidal concentration (MBC)

Spread plate technique was employed. A fresh solid medium was inoculated with inoculum from the least concentration that showed no visible growth and incubated for 24 h at 37ºC. The lowest concentration in which no growth occurs on the solid medium was accepted as the minimum bactericidal concentration. Table 1.3 shows that *Z. mauritiana*was active against *S. pyogenes*at 1 $mgml^{-1}$, botewere active against *E. coli* at 5 $mgml^{-1}$ but active against *S. aureus*only at 50 $mgml^{-1}$.The two fungal isolates*A. niger*and*C. albicans*were resistant. From Table 1.4 all theorganisms were susceptible to the antibiotics except *C. albicans*which was resistant. *Z. mauritiana*showed an MIC of 1 $mgml^{-1}$ against S.*pyogenes*, 5 $mgml^{-1}$ against *E. coli* and 40 $mgml^{-1}$ against *S. aureus*while*Z. spinachristi*showed the MIC of 5 $mgml^{-1}$ against *S. pyogenes*, 20 $mgml^{-1}$ against *E. coli* and 50 $mgml^{-1}$ against *S. Aureus*in. Table 1.5*Z. mauritiana*showed an MBC of 20 $mgml^{-1}$ against *S. pyogenes*, 30$mgml^{-1}$ against *E. coli* and 50 $mgml^{-1}$ against *S. aureus* while *Z.nummularia*showed the MBC of 30$mgml^{-1}$ against *S. pyogenes*, 40$mgml^{-1}$ against E*. coli* and 50$mgml^{-1}$ against *S. aureus*(Table 4).

Conclusion

The findings of this research work have shown clearly that the plants extracts are probably inactive against fungi and may not be useful in treating diseases of fungal origin. The extracts were active against the clinical isolates employed for this analysis. All the plants extracts were active against *S. pyogenes*an indication that the plant can be used to cure acute tonsillitis and sore throat caused by this bacterium. *Z. mauritiana*was active against *E. coli, S. pyogenes*and*S. aureus*while*Z. nummularia*was very active only against *S. pyogenes*but moderately active against the rest test organisms. *Z. mauritiana*showed stronger activity against the organisms compared with *Z. nummularia.*

The standard antibiotics used as control showed higher activity on the organisms than the extracts (Tables 1.3 and 1.4). This is not surprising because standard antibiotics are well refined industrial products so there is no doubt their activity will be more compared to crude extracts. If the extracts used in the present work are refined, more and better activity could be observed. The Minimum Inhibitory Concentration of the extracts against the organisms was 1 $mgml^{-1}$ against *S. pyogenes*and 5 $mgml^{-1}$ against *E. coli* while the Minimum Bactericidal Concentration was 5 and 20 $mgml^{-1}$, respectively against the organisms had similar results in their experiments involving some of these organisms. A cidal drug kills pathogens at levels only two or four times the MIC whereas a static drug kills pathogens at much higher concentrations. Some of the organic compounds detected in the extracts include tannins, saponins, resins, polyphenols and cardiac glycosides. These compounds have variously been reported to have antimicrobial activity and could be the reason for the activities recorded against these test organisms. Pants chemicals are thought to have the potentiality of useful drugs if properly harnessed.

The both plant extract are the founding good anti-oxidant activity. The current studies indicate that Ziziphusmauritiana and Ziziphus nummulariabrack, exerts a potential hepatoprotective activity against Ethanol-induced chronic toxicity,

comparable to silymarin. Ziziphusmauritiana and Ziziphus nummulariabrack, in a dose-dependent manner exhibited These studies indicate that the active constituents of Ziziphusmauritiana and Ziziphus nummulariabrack, should be further explored for its potential biological actions.

References

1. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. IV the Pune:NiraliPrakashan; 1996.
2. Vogel HG, Similarities between various systems of traditional medicine: Considerations for the future of ethnopharmacology. J. Ethnopharmacol., 1991, 35: 179-90.
3. Ramarao AV, Gurjar MK. Drugs from plant resources: an overview.Pharma.Times 1990; 22 (5):19-21
4. Handa SS. Plants as drugs. The Eastern Pharmacist 1991; XXXIV (397):79-85.
5. Jain SK, Ethnobotany and research on medicinal plants in India, CibaFound.Symp. 1994; 185:153-64.
6. Cragg GM, Boyd MR, Cardellina JH, N Newman DJ, SnaderKM,McCloud. Ethnobotany and drug discovery: the experience of the US National Cancer Institute developmental therapeutics Program, National Cancer Institute, Bethesda. Ciba Found. Symp., 1994; 185: 178-90.
7. Handa SS. Future trends of plants as drugs. Pharma. Times 1991; 23 (4):13-23.
8. Farnworth NR, A computerized data base for medicinal plants. The Eastern Pharmacist 1985; XXVIII (326):53-5.
9. Nearing M, The green pharmacy. Herbal medicines in modern usage. IDRC Rep., 1985; 14(1):10-1.
10. Mukherjee PK, Sahu M, Suresh B. Indian herbal medicines. The Eastern Pharmacist, 1998; XLI (490):21-3.
11. Tortora G.J, Grabowski S.R. Principles of Anatomy and Physiology. 8 thedHarper Collins College publishers inc. 1996; 824-3.
12. Robbins, Cotran, Kumar. Basic Pathology. 10 thed.
13. Liver complications.http://digestive.niddk.nih.gov/about/contact/htm.(15/8/2020)
14. Liver diseases.http://www.liverfoundation.org/(15/8/2020).
15. http://www.hepfi.org/(16/8/2020).
16. http://www.unos.org/(16/8/2020).
17. Avijeet J, Manish S, Lokesh D, Anurekha J, Rout SP, Gupta VB, Krishna KL. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica Roxb. Leaves. J Ethnopharmacol 2008; 115(1): 61-6.
18. Vipul Gujrati, Nilesh Patel, Venkat N Rao, K Nandakumar, TS Gouda,Md.Shalam S et al. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of Tylophora indica (Linn.) in rats. Ind J. Pharmacol 2007;39 (1):43-47.
19. Ramachandra SS, Absar AQ, Viswanath Swamy AHM, Tushar PPT, Prabhu K, Veeran GA. Hepatoprotective activity of Calotropis procera flowers against paracetamol-induced hepatic injury in rats. Fitoterapia 2007; 8: 451–454.
20. Tortora GJ, Grabowski SR. The digestive system (liver and gallbladder):

Principles of Anatomy and Physiology. 7th ed. New York: Harper Collins college publishers; 2002; 792-5.
21. www.enjoylongerhealth.com/liver-pic.html(9/8/2020)
22. www.arizonatransplant.com/healthtopics/liver.html(23/8/2021)
23. www.conatuspharma.com/liverfacts/html(10/9/2021)
24. Sembulingam K, PremaSembulingam. Liver and Gallbladder: Essentials of Medical Physiology. 3rd ed. New Delhi: Jaypee brother's medical publishers; 2004; 200-1.
25. Ghadi PS. Disorders of Liver: Pathophysiology for pharmacy. 2nd ed. Nashik: Career publications; 2000; 106-8,125-30.
26. Davidso. "Principle and Practice of Medicine", Churchill Livingstone Elsevier Science Ltd 1999; 19: 838-878
27. Gennavo AR, Remington. "The science and practice of pharmacy." Philadelphia: Lipincott Williams and Wilkins 2000;20:1088-1090.
28. Zimmerman HJ, Ishak KG. "Hepatotoxin injury due to drugs and toxins" London Churchill Livingstone 2002; 4: 622-24.
29. IshakKG.Theliver,"PathologyofDrugInducedandToxicDisease."Riddell RH, New York: Churchill Livingstone, 1982; 459.
30. Kirchain WR, Gill MA. "Drug – Induced Liver Disease" ,Pharmaco therapy a pathophysiological approach. London Appleton and Lange 1999;4:628-636.
31. Farell GC. "Drug –induced Liver Disease." Current Hepatology1991;11:95-110.
32. Shelly Lu, Neil Kaptowitz. "Drug–induced Hepatotoxicity." Current Hepatology 1989; 9:105-133.
33. Laurance DR, Bennett PN, Brown MJ. Clinical Pharmacology. Edinburg: Churchill Livingstone 1997; 8:589-590.
34. Harsh Mohan. "Text book of pathology." Jaypee brothers Medical Publishers (P) Ltd, New Delhi, 2000;4:599.
35. Balasubramaniyan V, Kalaivani Sailaja J, Nalini N. Role of leptin on alcohol-induced oxidative stress in swiss mice Pharmacological Research 2003; 47: 211–216.
36. Hemieda FA, Abdel-Hady el-SK, Elnga MA. Biochemical and histological studies on H2-receptor antagonist ranitidine-induced hepatotoxicity in rats. Ind J Exp Biol 2005; 43(9):782-5.
37. Kumar, Cotran. Robbins. "Basic Pathology", Saunders, the Curtis center, Philadelphia Pennsylvania 2004; 7:614.
38. Harsh Mohan. "Textbook of Pathology", Jaypee brothers Medical Publishers Ltd 2000; 4:602-603.
39. Cameron GR, Thomas JC and Karunarathe WAE. The pathogenesis of liver injury in carbon tetrachloride and ethanol poisoning. J. Path. Bact 1936; 41:297.
40. Handa SS and Sharma A. Hepatoprotective activity of Andrographolide from *Andrographis paniculata*against carbon tetrachloride. Ind. J. Med. Res 1990; 92: 276-92.
41. Shirwaiker A, Sreenivasan KK, Krishnan and BR and Kumar AV. Chemical investigation and anti hepatotoxic activity of the root bark of *Caparis spinosa. Fitoterapia*1996; 67 (3): 200-4.
42. Zimmerman MD and Hayman J. Function and integrity of the liver, In: Clinical diagnosis and management by laboratory methods In: Clinical diagnosis and management by laboratory methods. 17 th Ed. 1976;217-50.

43. Agarwal AK and Mehendale JK. Potentiation of carbon tetrachloride hepato toxicity and lethality by chlordecone in female rats. Toxicology 1983;26:231-42.
44. Dawkins MJR. J. Path. Bact.,1963;85:189.
45. Rang HR, Dale MM. "Pharmacology", Churchill Livingstone 2003; 5:391, 509, 546, 557.
46. Fan Sabrina, Weng Ching-Feng. Co-administration of Cyclosporine A Alleviates ethanol induced liver injury. World J Gastroenterol2005;11(10):1411-1419.
47. Shapiro Haim, Ashkenazi Michal, Weizman Nir, Shahmurov Mark Hussein Aeed, Bruck Rafael. Curcumin ameliorates acute ethanol induced hepatotoxicity. J of Gastroenterol &Hepato 2006; 21:358-366.
48. Hemieda Faried AE, Abdel Hady El-Sayed. Biochemical and histological studies on H2- receptor antagonist ranitidine-induced hepatotoxicity in rats. Ind J Exp Bio2005;43:782-785.
49. M Raza, M Ahmad, A Gado, OA Al-Shabanah. A comparison of hepatoprotective activities of amino guanidine and N-acetyl cysteine in rat against the toxic damage induced by azathioprine. Comparative BiochemPhysiol 2003; 134: 451–456.
50. Kapur V, Pillai KK, Hussain SZ and Balani DK. Hepatoprotective activity of Jigrine on liver damage caused by alcohol-carbon tetrachloride and paracetamol in rats. Ind. J. Pharmacol1994;26:35-40.
51. Saraswat B, Visen PKS, Dayal R, Agarwal DP and Patnaik GK. Protective action of ursolic acid against chemical induced hepatotoxicity in rats, Ind. J. Pharmacol. 1996; 28: 232-39.
52. Padma W, Suja V, Shyamala DCS and Prema. Hepatoprotective effect of Liv-52 on antitubercular drug-induced hepatotoxicity in rats. Fitoterapia 1998; 69(6): 520-22.
53. Harshmohan. The liver, biliary tract and exocrine pancreas. In: Text book of pathology, 4 th Ed, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi. 2002; 22-4 ,569-630.
54. Kind PRN and King EJ. Estimation of Plasma Phosphates by Determination of Hydrolysed Phenol with Antipyrine. J. Clin. Pathol.1954;322-30.
55. Klaassen CD and Plaa GL. Effect of CCl_4 on the Metabolism, Storage and Excretion of Sulfobromophthalein. Toxicol. Appl. Pharmacol 1968; 12:132-9.
56. Avadhoot Y and Rana AC. Hepatoprotective effect of Vitexnegundo against carbon tetrachloride induced liver damage. Arch. Pharm. Res. 1991; 14(1):96-8.